

SHORT COMMUNICATION

Megaselia scalaris (Diptera: Phoridae): an opportunistic endoparasitoid of the endangered Mexican redrump tarantula, *Brachypelma vagans* (Araneae: Theraphosidae)

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Abstract. Despite the importance of tarantulas in the areas of medicine and veterinary science, there is very little information on parasitoid-tarantula interactions. The present study describes the case of an endangered tarantula, *Brachypelma vagans* Ausserer 1875, infested by an endoparasitoid in the field. Using DNA barcoding, we identified the parasitoid as the phorid *Megaselia scalaris*. With more than 500 fly larvae inside the host, this particular infestation can be considered severe. The size range of the larvae indicates infestation by all three larval instars. We discuss the possible mechanism by which the parasitoid is attracted to the tarantula and make important recommendations regarding improvements in tarantula-rearing conditions. Finally, this case study exemplifies the efficiency of molecular technology for parasitoid identification.

Keywords: Spider, parasitism, DNA barcoding, humpbacked flies, larvae morphology

Current knowledge on tarantula parasites and parasitoids is very limited. This is surprising considering the popularity of these spiders as pets and in zoos (Saul-Gershenz 1996), their use in medical (Park et al. 2008; Machkour-M'Rabet et al. 2011) and veterinary applications (Pizzi 2009), and that several tarantula species are protected. Consequently, any additional knowledge associated with tarantula parasites/parasitoids is relevant and indispensable.

Parasitoids are organisms characterised by first instars that grow on or inside the host and always kill it as part of their life cycle (Godfray & Shimada 1999), usually attacking different developmental stages of their host. Many species of spider are parasitized by a variety of insects (Eason et al. 1967), most of which belong to the arthropod orders Hymenoptera and Diptera (Korenko et al. 2011), as well as some nematodes (Poinar 1985, 1987; Penney & Bennett 2006), and kleptoparasitic spiders (Hénaut et al. 2005). Considering only dipteran parasitoids, those in the family Acroceridae are the most representative (Schlinger 1993), although some species from the Tachinidae, Chloropidae, and Drosophilidae families also parasitize spiders (Eason et al. 1967; Disney 1994). The Phoridae family comprises over 3000 species of small humpbacked flies found worldwide and includes scavengers, herbivores, predators, and parasites/parasitoids (Boehme et al. 2010). Parasitoid species of these flies are reported to parasitize mainly spider egg sacs. For example, larvae of *Phalacrotophora epeirae* Brues 1902, feed on the egg mass of spiders of various families (Muma & Stone 1971; Hieber 1992; Guarisco 2001). In addition, parasitoids of the genus *Megaselia* have been associated with numerous families of spiders including Araneidae (Finch 2005), Theridiidae and Lycosidae (Rollard 1990).

Tarantulas belong to the family Theraphosidae comprising 947 species (Platnick 2014). Although reports of tarantula parasitoids are extremely rare, the most recognized species is *Pepsis* spp. (Hymenoptera: Pompilidae) (Vardy 2000, 2005; Costa et al. 2004). Pizzi (2009) mentions that ichneumonid ectoparasites (Hymenoptera) possibly lay their eggs on captive tarantulas and also refers to two nematode families: Mermithidae and Panagrolaimidae, which parasitize wild and

captive tarantulas respectively. Dermestid larvae (Coleoptera) parasitize captive *Brachypelma smithi* (Pickard-Cambridge 1897) specimens (Paré et al. 2001). Species of two Diptera families, Phoridae (Weinman & Disney 1997) and Acroceridae (von Eickstedt 1971, 1974; Cady et al. 1993), have also been reported as tarantula parasitoids.

Despite the high number of tarantula species in Mexico, only one study mentions the interaction between a parasitoid (*Pepsis* spp.) and a theraphosid spider (species of *Aphonopelma* Pocock 1901) (Punzo 2007). Of the 11 tarantula genera in Mexico (Platnick 2014), only *Brachypelma* Simon 1891 is protected under CITES (Appendix II). Throughout the last decade, efforts have been made to understand *Brachypelma* species and to contribute to their protection and conservation (e.g.: Machkour-M'Rabet et al. 2011, 2012; Vilchis-Nestor et al. 2013; Dor & Hénaut 2011, 2013; Dor et al. 2008, 2011).

A wild Mexican redrump tarantula, *Brachypelma vagans* Ausserer 1875 presented signs of weakness, leading to speculation that the spider was infested by fly larvae. After a short period of time, the tarantula died. No previous reports describe any manifestations or characteristics of a parasite infestation in this particular species of spider. Therefore, this occurrence presented a rare and exceptional opportunity to describe the case of an endoparasitoid infecting a protected species of tarantula.

The identification of a dipteran parasitoid, particularly as a larval instar, is problematical for the non-specialist taxonomist. DNA-based technology provides a possible solution to the problem of species identification. Hebert et al. (2004) developed an identification method known as "DNA barcoding", which uses part of the mitochondrial COI gene. This method is suitable for characterizing a large number of organisms (e.g.: Hebert et al. 2004; Prado et al. 2011), particularly parasitoids (Smith et al. 2007; Janzen et al. 2009; Zaldívar-Riverón et al. 2010), therefore, providing a unique opportunity to identify this specific tarantula parasitoid.

The aims of our study were i) to describe the manifestations presented by this spider during infestation and ii) to identify the endoparasitoid and describe the infestation.

The tarantula specimen was found in the village of “Laguna Guerrero” (Quintana Roo, Mexico) and taken to a laboratory maintained under standard conditions (25° C, 75% RH, natural light cycle). The tarantula was solitarily housed inside a plastic box (15 × 10 × 20 cm) to be reared for eventual reproduction. After a short period of time, the spider became inactive and showed no interest in food (adults of *Tenebrio molitor* Linnaeus 1758, Coleoptera: Tenebrionidae). Eventually, the tarantula stopped moving, as in pre-moulting behaviour, and its abdomen became abnormally distended. After two days, the tarantula adopted a huddled up position (all legs adducted, placing the tarsal tips under the sternum) and died. It was placed in 96% ethanol and after a few days, numerous dipterous larvae, assumed to have emerged from the spider, were observed in the alcohol (larvae deposited in the Zoological Museum of ECOSUR, Chetumal, Mexico).

All the larvae were collected from the alcohol and the tarantula was dissected to remove any remaining individuals from the carcass. The larvae were counted and their length measured (Stemi DV4 Zeiss stereomicroscope with measuring eyepiece, 32X magnification) to determine the larval instar. Twenty-five first and second-instar larvae were sent to the “Laboratorio de Microscopía Electrónica de Barrido” (Scanning Electron Microscopy Laboratory) at ECOSUR (Tapachula, Mexico) to confirm the presence of different larval instars and identify their morphological characteristics. Due to damage, third-instar larvae were not sent to the microscopy laboratory. Larvae were washed with 100% ethanol using a fine brush, submitted to several baths of 100% ethanol to remove any external elements and then dehydrated in 100% ethanol for 12 hours. They were subjected to critical point drying under CO₂ before being attached to double-sticky tape on aluminum stubs and coated with palladium-gold (20 nm thick) in a sputter-coating apparatus (Denton Vacuum, Desk II) for viewing under a scanning electron microscope (Topcon, SM-510).

For the molecular analysis by “DNA barcoding”, five larvae were placed in a lysis 96-well plate with a drop of 96% ethanol. Genomic DNA was extracted from larval tissue and the extraction process was conducted following Montero-Pau et al. (2008). Amplification and sequencing of the DNA followed the protocols of Prado et al. (2011). Sequences and all collateral data from specimens are available on BOLD website (www.boldsystems.org) in the project entitled “PARTA”.

Using the tools provided by BOLD-IDS, the obtained DNA barcode permitted identification to order and family level: Diptera and Phoridae respectively. The BLAST® tool from GenBank was then used for species level identification, providing a match with *Megaselia scalaris* Loew 1866 (99% similarity).

The *B. vagans* individual presented a high level of parasitism, hosting 524 larvae from a wide range of sizes representing the three larval-instars. The size frequency analysis suggests that second-instar individuals were dominant (Fig. 1). Following Sukontason et al. (2002) and Boonchu et al. (2004), the binomial distribution of size frequencies (Fig. 1) and the larvae ultrastructures (Fig. 2A–F) were used to determine the size range for each larval-instar. The size of second-instar larvae ranged from 1.0 mm to 3.5 mm ($n = 466$; 88.9% of total larvae), with a mean of $2.08 \text{ mm} \pm 0.02$ ($\pm \text{SE}$) (Fig. 2A). The characteristic ultrastructures of the spiracular slits of the posterior abdominal spiracles (Fig. 2B) and the triangular-shaped labium, typical of second instar larvae, were identified (Fig. 2C). Some individuals were first-instar (from 0.5 mm to 0.9 mm; $n = 55$; 10.5% of total larvae) with a mean size of $0.65 \text{ mm} \pm 0.014$ (Fig. 2D). These larvae showed rudimentary posterior abdominal spiracles that presented a broad-based posterior spiracular hair (Fig. 2E) and a characteristic bi-lobed labium (Fig. 2F). There were only three third-instar individuals (from 3.6 mm to 3.8 mm; $n = 3$; 0.6% of total larvae) with a mean size of $3.7 \text{ mm} \pm 0.058$. As these larvae were damaged, no morphological characteristics were identified.

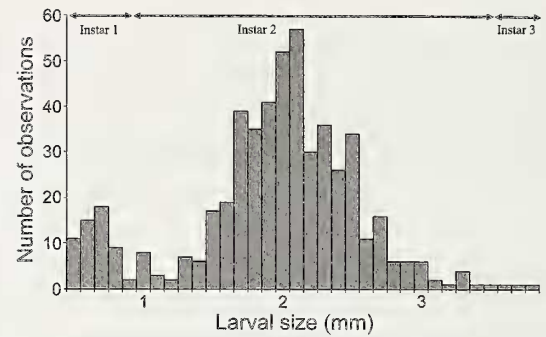


Figure 1.—Frequency of larval sizes (mm) for the endoparasitoid *Megaselia scalaris* (Diptera: Phoridae) taken from a specimen of *Brachypelma vagans* (Araneae: Theraphosidae).

Megaselia scalaris is a cosmopolitan phorid fly with larvae that feed on a high diversity of decaying organic material, making this species a facultative predator, parasite, and parasitoid in invertebrate laboratory colonies (Costa et al. 2007; Disney 2008).

Megaselia is known to parasitize theraphosid spiders in Colombia (Weinmann & Disney 1997) and spiders of the genus *Theraphosa* Thorell 1870 in French Guiana (Marshall & Uetz 1990). However, this is the first report of a living endangered Mexican tarantula species hosting a parasitoid in the wild. Although no observations were made, *M. scalaris* adults were probably attracted by the accumulated remains (prey and moult) in the tarantula burrow (Machkour-M'Rabet et al. 2007). These flies became parasitoids of the living spider by using the book lung as an entrance point and subsequently penetrating the opisthosoma and internal organs (Pizzi 2009). This hypothesis is substantiated by several studies that describe phorid adults feeding on the spider's prey (Sivinski & Stowe 1980; Weinmann & Disney 1997) and being attracted to the stabilimentum of the spider's web by the strong smell of decaying matter (Hénaut et al. 2010). Weinmann & Disney (1997) reported the presence of phorid larvae on living specimens of two theraphosid species, *Megaphobema robustum* Ausserer 1875 and *Pamphobeteus* Pocock 1901, in Colombia. The phorid species were identified as *Megaselia dimorphica* Disney 1997 and *Megaselia praedafura* Disney 1997. Marshall (pers. obs. in Marshall & Uetz 1990) reported a *Megaselia* fly associated with *Theraphosa* spiders in French Guiana. In another study, Pérez-Miles et al. (2005) suggest that the silk that covers the burrow entrance during the day provides protection against parasitoids.

The tarantula's death was not unexpected as the level of infestation, (over 500 larvae) was considered very high. One study reports 138 specimens of *M. scalaris* on a piece of sardine (Moretti et al. 2009). This number of larvae is not exceptional when considering the high fecundity of *M. scalaris* females that can lay up to 600 eggs (references in Disney 2008). The high level of infestation by different fly instars could be the result of a single female oviposition over a period of several weeks, or ovipositions from different females at different times.

Parasitoidism by phorid flies poses a potential risk to tarantula breeding for pets or scientific use. Therefore, it is crucial that these spiders are adequately managed and protected. Constant cleaning, maintaining optimal temperature and humidity, control of new individuals through a quarantine period, and the mechanical protection of spiders from parasitoid arrival would substantially reduce the risk of infestation by this dipteran on *Brachypelma* spp. Furthermore, the identification of a parasitized *B. vagans* in the field highlights the potential risk for natural populations of these endangered tarantulas. More research is necessary to evaluate the impact of fly parasitoids on wild tarantula populations.

Megaselia scalaris was successfully identified using DNA barcoding. Because morphological determination to the species level is

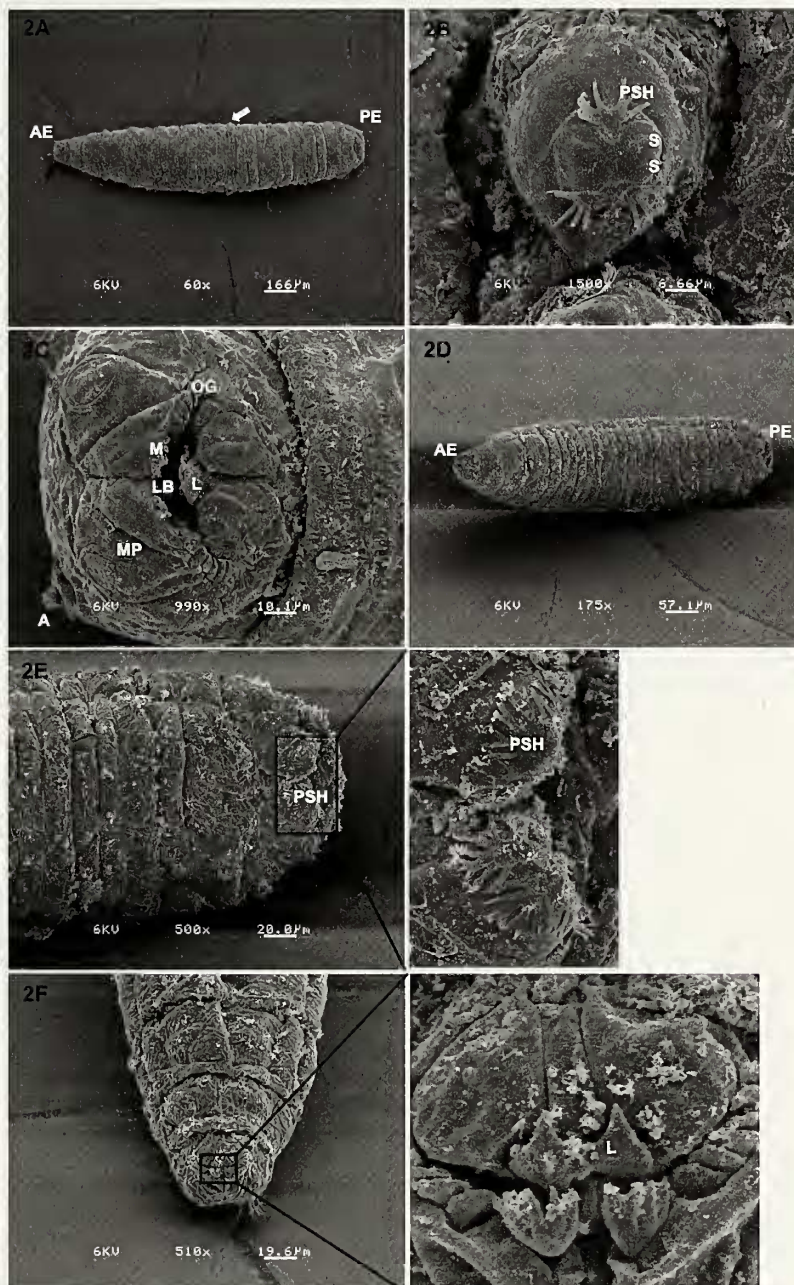


Figure 2.—Scanning electron micrographs of *Megaselia scalaris* (Diptera:Phoridae) larvae taken from a specimen of *Brachypelma vagans* (Araneae: Theraphosidae). (A) Ventral view of the entire body of a second-instar larva with anterior end (AE) and posterior end (PE). White arrow indicates short spinous process and black arrow shows cephalic segment. (B) Posterior spiracular disc of a second instar larva with its two straight slits (S) for each expanded end, and the posterior spiracular hairs (PSH). (C) Frontal view of the cephalic segment of a second-instar, illustrating the antenna (A), labium (L), labrum (LB), oral groove (OG), mouth hooks (MH) and maxillary palp complex (MPC). (D) Ventral view of the entire body of a first-instar with anterior end (AE) and posterior end (PE). (E) Broad-based posterior spiracular hairs of a first-instar (PSH). (F) Frontal view of the cephalic segment illustrating the bi-lobed labium (L) of a first-instar.

especially difficult for larvae and pupae, the use of a DNA-based method is an excellent alternative. We hope that this DNA barcoding technique will become a straightforward laboratory routine for non-specialists in molecular ecology in order to rapidly resolve issues of specimen identification.

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